Pathogenesis of Hypoglycemia in Insulinoma Patients

Suppression of Hepatic Glucose Production by Insulin

ROBERT A. RIZZA, MOREY W. HAYMOND, CARLOS A. VERDONK, LAWRENCE J. MANDARINO, JOHN M. MILES, F. JOHN SERVICE, AND JOHN E. GERICH

SUMMARY

To determine the mechanism by which hyperinsulinemia causes hypoglycemia in insulinoma patients, rates of glucose production and utilization, and circulating levels of insulin, glucagon, alanine, lactate, and glycerol were measured in 6 insulinoma patients during development of fasting hypoglycemia and in 8 normal volunteers studied over an identical interval. Initially, insulinoma patients had a greater plasma insulin (42 ± 9 versus 15 ± 1 μU/ml) and glucagon levels (214 ± 31 versus 158 ± 21 pg/ml) than normal subjects, $P < 0.05$, but their plasma glucose levels (81 ± 4 mg/dl) and rates of glucose production and utilization (1.71 ± 0.08 and 1.74 ± 0.08 mg/kg · min, respectively) were not significantly different from those of normal subjects (93 ± 2 mg/dl, 1.93 ± 0.11, and 1.92 ± 0.13 mg/kg · min, respectively). During a subsequent 8-h fast, glucose production and glucose utilization decreased in both groups, but more markedly in insulinoma patients. Since glucose utilization exceeded glucose production to a greater extent in insulinoma patients than in normal subjects, plasma glucose decreased to 44 ± 3 mg/dl in insulinoma patients, but only to 84 ± 1 mg/dl in normal subjects ($P < 0.001$). Glucose utilization in insulinoma patients never exceeded that of normal subjects. These results demonstrate that fasting hypoglycemia in the insulinoma patients is usually due to suppression of glucose production rather than to acceleration of glucose utilization, as is widely thought. A direct effect of insulin on the liver is probably responsible, since circulating levels of gluconeogenic precursors are normal and since plasma glucagon increases during development of hypoglycemia in insulinoma patients. **DIABETES *30*:377–381, May 1981.**

The development of fasting hypoglycemia in insulinoma patients is due to excess insulin. Most textbooks currently attribute this hypoglycemia predominantly, if not exclusively, to acceleration of glucose utilization by insulin.[1–3] However, insulin can decrease plasma glucose levels in man by suppressing glucose production, as well as by augmenting glucose utilization.[4–6] Since rates of glucose production and utilization have not as yet been measured in insulinoma patients, the actual pathogenic significance of alterations in these processes remains to be established. Therefore, to determine the mechanism responsible for hypoglycemia in these patients, rates of glucose production and utilization and circulating levels of insulin, glucagon, and potential gluconeogenic precursors were measured in 6 insulinoma patients during the evolution of fasting hypoglycemia and in 8 normal volunteers studied over an identical interval. The results of these studies indicate that fasting hypoglycemia in insulinoma patients is usually due to suppression of glucose production rather than to acceleration of glucose utilization.

METHODS AND MATERIALS

Informed written consent was obtained from 6 patients (5 M, 1 F, aged 39–69 yr, mean 54 ± 2 yr) referred to the Mayo Clinic, Rochester, Minnesota for the diagnosis of fasting hypoglycemia, and from 8 normal volunteers (5 F, 3 M, aged 18–51 yr, mean 28 ± 5 yr). All subjects were within 15% of their ideal body weight and had no family history of diabetes mellitus. The insulinoma patients had had excessive plasma insulin concentrations (>15 μU/ml) documented coincident with hypoglycemia (plasma glucose <40 mg/dl) during a supervised diagnostic fast. None had insulin antibodies. Duration of symptoms ranged from 1 to 5 yr, mean 3.0 ± 0.6 yr. Subsequent to the present studies, all patients had restoration of euglycemia after surgical removal of a single pancreatic islet cell adenoma.

On the morning of the study, the insulinoma patients and normal subjects were admitted to the outpatient facility of the Mayo Clinic General Research Center between 7:00 and 8:00 a.m. All were placed at bed rest and remained supine thereafter. The normal subjects were in the postabsorptive

From the Endocrine Research Unit, Departments of Medicine, Pediatrics, and Physiology, Mayo Medical School and Mayo Clinic, Rochester, Minnesota. Address reprint requests to John E. Gerich, Endocrine Research Unit, Department of Medicine and Physiology, Mayo Clinic, Rochester, Minnesota 55901.
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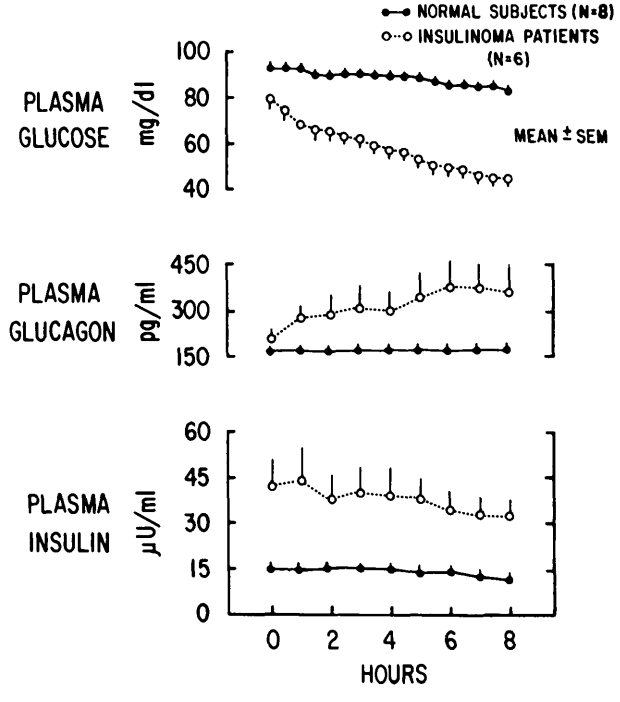
state, having last eaten 10–12 h earlier; the insulinoma patients had been given a 300-cal snack containing 30–40 g carbohydrate 3–4 h earlier. Both groups remained fasting throughout the period of study. An antecubital vein of each arm was cannulated with an 18-gauge catheter; one arm was used for infusion of isotope, and the other for intermittent blood sampling. A primed (11 μCi) continuous (0.11 μCi) infusion of [3-^{3}H]-glucose (New England Nuclear, Boston, Massachusetts) was begun for isotopic determination of rates of glucose production and utilization.[7] Two hours were allowed for isotopic equilibration before all experiments. Blood samples were obtained over the next 8 h for determination of plasma glucose (glucose-oxidase method, Yellow Springs Instrument A23); glucose specific activity[7] (30-min intervals); insulin[8] and glucagon[9] (60-min intervals); and glycerol,[10] lactate,[11] and alanine[12] (120-min intervals).

Rates of glucose production and utilization were calculated using the equations of Steele et al.[13] as modified by DeBodo et al.[14] Glucose clearance was calculated by dividing the rate of glucose utilization by the appropriate plasma glucose concentration.[15] Net cumulative glucose balance was calculated as the algebraic sum of glucose production and glucose utilization over each 30-min interval. The validity of the use of [3-^{3}H]-glucose as a nonrecycling trace has been discussed in detail.[16] All data in the text and figures are expressed as mean ± SEM. Statistical significance was evaluated using Student's two tailed paired and, when appropriate, non-paired *t* tests.[17]

RESULTS

Plasma glucose, insulin, glucagon, glycerol, alanine, and lactate (Figure 1 and Table 1). At initiation of the study, the plasma glucose concentration of insulinoma patients (81 ± 4 mg/dl) was slightly, but not significantly, less than that of the normal subjects (93 ± 2 mg/dl). However, both plasma insulin (42 ± 9 μU/ml, 18–70 μU/ml) and glucagon (214 ± 31 pg/ml) concentrations of the insulinoma patients were significantly greater than those of the normal subjects (15 ± 1 μU/ml and 158 ± 21 pg/ml, respectively, $P < 0.05$). During the 8-h study period, plasma glucose decreased to 44 ± 3 mg/dl in the insulinoma patients, but only to 84 ± 1 mg/dl in the normal subjects ($P < 0.001$). Plasma insulin decreased slightly in both groups ($P < 0.05$) but remained greater in the insulinoma patients (37 ± 8 versus 11 ± 1 μU/ml, $P < 0.01$). Plasma glucagon increased to 356 ± 99 pg/ml ($P < 0.05$) in the insulinoma patients, but did not change in the normal subjects. Throughout the study, plasma glycerol, alanine, and lactate concentrations of the insulinoma patients were not significantly lower than those of the normal subjects (Table 1).

FIGURE 1. Plasma glucose, glucagon, and insulin concentrations in insulinoma patients and normal subjects during an 8-h fast.



Rates of glucose production, utilization, clearance, and cumulative glucose balance (Figure 2). At initiation of the study, rates of glucose production (1.71 ± 0.08 mg/kg · min), utilization (1.74 ± 0.08 mg/kg · min), and clearance (2.20 ± 0.13 ml/kg · min) in the insulinoma patients were not significantly different from the corresponding rates in the normal subjects (1.93 ± 0.10 and 1.92 ± 0.13 mg/kg · min, and 2.07 ± 0.14 ml/kg · min, respectively). During the 8-h study period, rates of glucose production and glucose utilization decreased in both groups ($P < 0.01$), but both glucose production and glucose utilization decreased to a greater extent in the insulinoma patients. At the end of 8 h, glucose production and utilization averaged 1.21 ± 0.10 and 1.28 ± 0.10 mg/kg · min, respectively, compared with corresponding values of 1.60 ± 0.11 and 1.62 ± 0.12 mg/kg · min in the normal subjects ($P < 0.05$). At no time did the rate of glucose utilization exceed that of the normal subjects. Rates of glucose utilization and production were closely matched throughout in the normal subjects, resulting in a slightly negative net cumulative glucose balance (−8.7 ± 2.7 mg/kg); in contrast, in the insulinoma patients glucose utilization consistently exceeded glucose production, resulting in a more negative net cumulative glucose balance (−36.6 ± 5.4 mg/kg, $P < 0.001$). Glucose clearance remained at basal rates in the normal subjects, but increased in the insulinoma patients to 2.86 ± 0.25 ml/kg · min at the end of 8 h ($P < 0.01$) despite the fact that their plasma insulin concentrations had decreased.

DISCUSSION

Fasting hypoglycemia associated with absolute or relative hyperinsulinemia is characteristic of patients with an insulinoma. This hypoglycemia has generally been attributed to an acceleration of glucose utilization by insulin.[1–3] However, this premise has not been directly verified, since rates of glucose production and utilization have not previously been measured in such patients. Insulin can decrease plasma glucose levels in man both by suppression of glucose production and by augmenting glucose utilization.[4–6] Thus, either or both of these mechanisms might be involved in the development of hypoglycemia in patients with hyperinsulinemia.

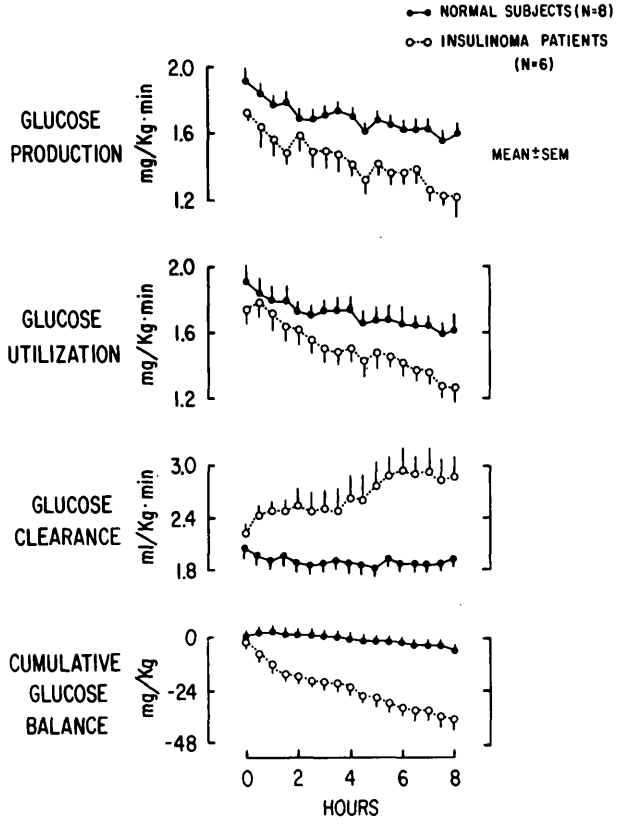
The results of the present studies, in which rates of glucose production and utilization were determined during the evolution of fasting hypoglycemia in the insulinoma patients, indicate that suppression of glucose production rather than acceleration of glucose utilization is the primary

TABLE 1
Plasma concentrations of glycerol, alanine, and lactate

	Time (min)				
	0	120	240	360	480
Glycerol (μM)					
Insulinoma patients	122 ± 37	110 ± 29	101 ± 16	106 ± 12	119 ± 11
Normal subjects	61 ± 6	60 ± 9	62 ± 9	76 ± 7	70 ± 5
Alanine (μM)					
Insulinoma patients	495 ± 72	416 ± 68	345 ± 64	309 ± 73	295 ± 45
Normal subjects	308 ± 49	350 ± 43	309 ± 46	296 ± 45	310 ± 44
Lactate (μM)					
Insulinoma patients	1315 ± 152	1384 ± 146	1034 ± 131	979 ± 152	984 ± 129
Normal subjects	915 ± 105	833 ± 94	764 ± 5	709 ± 60	637 ± 44

mechanism responsible for the hypoglycemia in such patients; thus, during the development of hypoglycemia, both glucose production and glucose utilization decreased to a greater extent in the insulinoma patients than in normal subjects studied over an identical interval. Since the suppressed rate of glucose utilization consistently exceeded the suppressed rate of glucose production in insulinoma patients, glucose balance became progressively more negative, resulting in a continued decrease in plasma glucose levels. It is worth noting that the insulinoma patients as a group tended to be older than the normal volunteers. However, since the results obtained in the two oldest normal volunteers (males aged 45 and 51 yr), whose age was similar to the insulinoma patients, did not differ from those in the other normal volunteers, it seems unlikely that differences in age could account for the differences in glucose flux rates in the insulinoma patients and the normal volunteers.

FIGURE 2. Rates of glucose production, utilization, and clearance and the cumulative glucose balance in insulinoma patients and normal subjects during an 8-h fast.



It should be pointed out that, although glucose utilization decreased during the development of hypoglycemia, it may have been inappropriately high for the prevailing plasma glucose concentration and, thus, may have contributed to the development of hypoglycemia. Nevertheless, the suppression of glucose production would still appear to be quantitatively the more important factor since, without suppression of glucose utilization, a relative increase in glucose production would not by itself cause hypoglycemia. This conclusion may not necessarily apply to the insulinoma patient with marked hyperinsulinemia, since under such circumstances hypoglycemia may be due to both decreased glucose production as well as increased glucose utilization.

Suppression of glucose production in the insulinoma patients may have been due to a variety of insulin-mediated mechanisms, e.g., limitation of substrate availability,[18] suppression of glucagon secretion[19] or antagonism of its action on the liver,[20] or inhibition of gluconeogenic or glycogenolytic enzyme activity.[21] The increases in plasma glucagon observed during the development of hypoglycemia, the normal circulating levels of alanine, glycerol, and lactate throughout the study, and the fact that the insulinoma patients had consumed a meal 3–4 h before the study argue against limitation of substrate availability, suppression of glucagon secretion, and glycogen depletion as possible mechanisms. Thus, the results of the present studies suggest that a direct effect of insulin on the liver was responsible.

The technique employed in the present studies does not permit evaluation of the individual contributions of glycogenolysis and gluconeogenesis to overall glucose production. But, since gluconeogenesis generally accounts for only 20–25% of glucose production in the early postabsorptive state,[22] and since glucose production was suppressed almost 40% in the insulinoma patients, suppression of glycogenolysis must have been involved to some extent. That this may have been the predominant mechanism is suggested by observations that glycogenolysis appears to be more readily suppressed by insulin than is gluconeogenesis.[28]

Surprisingly, glucose utilization in the insulinoma patients was not increased despite plasma insulin concentrations that were two- to threefold greater than normal. Acute infusion of insulin producing plasma insulin concentrations comparable to those observed in the insulinoma patients

has been shown to result in an increase in glucose utilization as well as suppression of glucose production in normal man.[6] Conceivably, the lack of augmented glucose utilization in the insulinoma patients may have been due, in part, to the fact that a substantial portion of the circulating immunoreactive insulin may have consisted of biologically inactive peptides (e.g., proinsulin).[24,25] Moreover, chronic hyperinsulinemia could have caused "downregulation" of insulin receptors;[26] insulinoma patients have been reported to have decreases in the number and affinity of insulin receptors on their monocytes.[27]

The suppression of hepatic glucose production observed in the insulinoma patients in the absence of accelerated glucose utilization may be explained by the fact that the liver is exposed to portal venous insulin concentrations that are 3–5 times greater than the arterial insulin concentrations to which peripheral tissues are exposed.[28] Indeed, recent dose-response studies in man indicate that suppression of hepatic glucose production is more sensitive to insulin than is stimulation of peripheral glucose utilization and that, under euglycemic conditions, glucose production may be totally suppressed at plasma insulin concentrations as low as 50 μU/ml.[29]

It should be pointed out that the failure to demonstrate augmented glucose utilization in the insulinoma patients could also have been due to the somewhat reduced plasma glucose concentrations, since glucose utilization in vivo is dependent in part on the plasma glucose concentration.[30] Although this probably explains the suppression of glucose utilization during the evolution of hypoglycemia in the insulinoma patients, it is unlikely to have been responsible for the lack of augmented glucose utilization at initiation of the study when plasma glucose levels were near normal. This is evident from the fact that glucose clearance, a mathematical expression with which the rate of glucose utilization is normalized for the mass action effect of glucose, was virtually identical in the insulinoma patients and the normal subjects at the initiation of the study. Subsequently, however, as plasma glucose concentrations decreased, glucose clearance increased in the insulinoma patients despite the fact that plasma insulin concentrations decreased. This was probably due to the obligatory glucose utilization by insulin-dependent tissues such as brain and erythrocytes and indicates, as had been predicted from studies in dogs,[30] that when plasma glucose decreases below the euglycemic range in man, glucose clearance is no longer independent of plasma glucose concentration. The concept of glucose clearance, which assumes such independence, may therefore not be valid under hypoglycemic conditions.

In conclusion, in the present studies the primary cause of fasting hypoglycemia in insulinoma patients was suppression of glucose production rather than acceleration of glucose utilization as is widely presumed. The suppression of glucose production can be most readily explained by a direct hepatic effect of insulin to inhibit glycogenolysis and gluconeogenesis. The present studies also provide additional evidence for the central role of the liver in human glucose homeostasis by demonstrating that endogenously secreted insulin may markedly alter hepatic glucose metabolism without directly affecting peripheral glucose metabolism.

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